A Scanning Electron Microscopic Study of Cell Attachment to Biodegradable Polymer Implants

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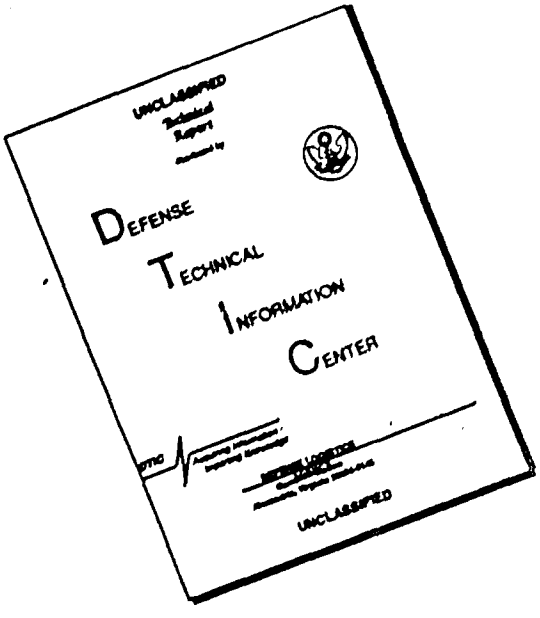

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The biodegradable polymers, polylactide (PLA) and polyglycolide (PGA), are currently being studi as carriers for bioactive bone regeneration compounds. The inclusion of osteoinductive substances in PLA-PGA copolymer alloplastic implants has been shown to enhance the repair of osseous defects. The purpose of this study was to examine, by SEM, the relationship of the biodegradation of the polymer to the attachment of cells and tissue matrix to the implant. Three groups of polymer were studied: 1) 50:50 PLA-PGA copolymer, 2)PLA-PGA copolymer with hydroxyapatite (HA) and 3) PLA-PGA copolymer with autolyzed, antigen-extracted (AA) bone particles. Four mm polymer discs were surgically implanted into the pectoralis muscles of rats in nylon baskets. At 7, 14, and 21 days post-implantation, the baskets were removed and the contents prepared for SEM. Results showed that at one week, implants are coated primarily with red and white blood cells, in a fibrinoid clot. Degradation of the polymers is evidenced by irregular enlarging of polymer surface pores. At two and three weeks, the implants became lobular and then fibrinoid as degradation progressed through the body of the

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19) implant disc. Inflammatory cell and red blood cell adhesions were increasingly replaced by fibroblasts and collagen matrix deposition. Degradation seemed to proceed faster with AA-copolymer than HA-copolymer implants. Plain copolymer discs degraded the slowest, being the most dense and least porous initially. As degradation progressed, AA and HA particles were exposed to the extracellular environment. However, the lack of cell or tissue adhesions to them, suggest that degradation is more influenced by the fluid environment than by direct cell attachment. Furthermore, degradation may inhibit direct cell attachment to some extent.

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SEM of PLG polymer - cell attachment

ABSTRACT

The biodegradable polymers, polylactic acid (PLA) and polyglycolic acid (PGA) are currently being studied as carriers for bioactive bone regeneration compounds. The inclusion of osteoinductive substances in poly- (DL, lactide-co-glycolide) copolymer alloplastic implants has been shown to enhance the repair of osseous defects. The purpose of this study was to examine, by SEM, the attachment relationship of biodegradable polymer implants to cells and tissue matrix. Three groups of copolymer implants were studied: 1) plain 50:50 PLA-PGA copolymer, 2) PLA-PGA copolymer with hydroxyapatite (HA) and 3) PLA-PGA copolymer with autolyzed, antigen-extracted (AA) bone particles. Polymer discs were surgically implanted into the pectoralis muscles of rats. At 7,14, and 21 days post-implantation, the baskets were removed and the contents prepared for SEM. Results showed that at one week, implants were coated primarily with red and white blood cells in a fibrinoid clot. Degradation of the polymers was evidenced by irregular enlarging of polymer surface pores. At two and three weeks, polymers became lobular and then fibrinoid as degradation progressed . Inflammatory cell and red blood cell adhesions were increasingly replaced by fibroblasts and collagen matrix deposition. As polymer degradation progressed, AA and HA particles were exposed; however, the lack of cell or tissue adhesion in these areas suggest that degradation may be more influenced by the fluid

environment than by direct cell attachment. Furthermore, degradation may inhibit direct cell attachment.

Keywords: Scanning electron microscopy, cell attachment, biodegradation, poly(DL, lactide-co-glycolide), implants.

INTRODUCTION

Biodegradable polymers have become increasingly popular for medical applications since the introduction of biodegradable suture material. The alpha-hydroxypolyesters, polyglycolic acid (PGA) and polylactic acid (PLA), have been shown to fulfill the requirements of biocompatibility and biodegradation for a variety of applications.(1-3) Current development of these versatile materials at the U.S. Army Institute of Dental Research focuses on their use for sustained antibiotic delivery, bone fracture stabilization, moldable osseous repair materials and as vehicles for delivery of bone inductive substances at sites of osseous defects. (4-6) Both PLA and PGA undergo biodegradation primarily through spontaneous hydrolytic scission which may be mediated by a variety of proteolytic enzymes. (1,7) Through this process, both lactic acid and glycolic acid, the respective degradation products of PLA and PGA, are formed in concentrations compatible with normal mammalian metabolism. While many biocompatibility studies have examined extent of tissue necrosis or degree of inflammatory response adjacent to implanted materials, the process of cell attachment in the face of biodegradation has seldom been evaluated. (8,9) The changes occurring during this dynamic period at the interface between the

implant and its degradative products and the surrounding cellular milieu have been difficult to study due to the artifactual effects of routine tissue processing for microscopy. This study examined, by scanning electron microscopy, the relationship between polymer biodegradation and attachment of cells and tissue matrix as a function of healing. A PLA-PGA copolymer incorporating either osteoconductive hydroxyapatite (HA) or osteoinductive autolyzed, antigen-extracted (AA) bone particles, both currently being evaluated as alloplastic bone repair materials, were examined.

MATERIALS AND METHODS

Biodegradable copolymer discs were fabricated by first solubilizing 50:50 molar poly (DL, lactide-co-glycolide) (Southern Research Institute,Birmingham, Ala.) in acetone. Either AA bone particles (prepared from Macaca fascicularis (cynomolgus) non-human primate according to a modification of the method of Marshall Urist) (Fig. 1) or HA (Interpore, Irving, Ca.) (Fig. 2) was added to the copolymer in a 1:1 weight ratio. Polymer was poured into petri plates and vacuum cured at 25 °C. In addition, 50:50 poly(DL, lactide-co-glycolide) copolymer without AA bone or HA was similarly prepared. Discs of 4 mm diameter and 1.5 mm thickness were cut and packaged in nylon mesh baskets which facilitated implantation and retrieval. Discs were implanted into surgically created pouches within the pectoralis muscle of 27, 12 - 15 week old male Long-Evans rats.* Three rats were implanted with each of the test polymer mixtures.

* NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) have been observed in conducting this research.

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There were three retrieval times per implant type. Samples were recovered at one, two and three weeks post-implantation, and were fixed for two hours in Karnowsky fixative (4% paraformaldehyde: 1% glutaraldehyde in 0.2M sodium cacodylate buffer, pH 7.4), washed in 0.1M buffer, post-fixed in 1% osmium tetroxide for one hour, *en bloc* stained with 1% uranyl acetate for 12 hours at 4 °C, dehydrated through graded ethanol to hexamethyldisilazane for 5 minutes and air dried. Samples were finally gold-palladium sputter- coated and photographed in an AMRAY 1645 turbo-scanning electron microscope.

RESULTS

At one week, implants showed a patchy fibrinoid coating containing a mixture of red blood cells, white blood cells and platelets (Fig.3). In some instances, platelets showed direct adhesion to the implant surface (Fig.4). Prior to implantation, cross-sectioned plain copolymer (i.e. without HA or AA bone particles) disks revealed internal porosity with an average pore diameter of 0.15μm (Fig. 5). Early degradation of the implants was evidenced by the irregular enlarging of polymer porosity (Fig.6). Scattered surface cratering was noted on the plain copolymer implants (Fig.7).

As degradation progressed through the body of the implant, the polymer developed a lobular appearance at two weeks and a more fibrinoid appearance by three weeks (Fig. 8,9). At two and three weeks, the deposition of first a gelatinous substance and later a loose collagenous matrix onto the degrading implant, made distinguishing between polymer and tissue matrix increasingly

difficult. Mixed aggregates of erythrocytes, leukocytes, apparent macrophages and fibroblasts were noted, but seldom directly attached to either the polymer or exposed particles of HA or AA bone repair material (Fig.10,11).

Due to the lack of particulate inclusions, the plain polymer was the most dense and least porous. It, therefore, seemed to initially degrade slower than the implants containing either AA or HA particles; however, this was a subjective observation. By three weeks, the surface of the plain polymer implants appeared similar to the HA- and AA-polymer implants (Fig. 12,13) .

DISCUSSION

A problem in the use of PLA-PGA copolymer has been variations in the degradation rate. It has been recognized that differences in the molar ratio of PLA to PGA significantly affect degradation variance.[10,11] Yet even with the same molar ratio of constituents, different studies have observed differing degradation rates.(10,12) In a study of tissue response to 50:50 PLA-PGA microcapsules, Visscher et al observed no significant degradation until 4 or more weeks of implantation.[12] Despite the fact that the mass of the disk implants used in this study was many times greater than that of Visscher's microcapsules, significant degradation was observed by three weeks. Cross-sectioned disks one week postimplantation revealed an increase in the pore diameter of nearly ten-fold (fig. 5 and 6). This suggests that the initial phase of degradation involves fluid imbibition, just as the pores of a sponge fill with water. This concept is supported by Kronenthal, who states

that hydration, or water absorption, is the first stage of degradation for many polymers.(13) This is followed by loss of strength due to cleavage of backbone covalent bonds. Next, as loss of mass integrity occurs and covalent bonds are further broken, molecular coherence is lost, and the mass becomes friable or gelatinous. Finally, removal of degraded polymer may occur either by phagocytosis or by solubilization of low molecular weight fragments into the intercellular fluid. Gilding suggests that the degradation of PLA and PGA occurs nearly entirely by water absorption and simple hydrolysis.(14) Surface changes at one week were not as dramatic as internal changes at this early time. However, by two weeks, large surface channels were apparent and appeared to communicate with the internal pores. As particles of hydroxyapatite and bone became increasingly exposed between two and three weeks, the polymer disks became soft and crumbly just as described by Kronenthal. By this time, a thin connective tissue capsule surrounded the implant within the nylon basket, and the distinction between invading stroma and degrading implant were difficult. This study did not follow the degradation process to the ultimate end. According to Miller (10) and Visscher (12) macrophages and foreign body giant cells play a late role in the final degradation of the polymer. Such cell types were not particularly prominent during the period of this study.

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CONCLUSIONS

1) Lack of cell or tissue adhesion to the degrading polymer and exposed AA and HA particles suggests that degradation is more influenced by the fluid environment than by direct cell attachment. Furthermore, polymer degradation may inhibit direct cell attachment to some extent. This may occur as the dissolution phase of degradation ensues with the local accumulation of lactic and glycolic acids as well as other metabolic enzymes.

2) The structural configuration of the bone repair material incorporated within the copolymer implant seems to affect polymer degradation, and possibly cell attachment to the implant. It appeared that the irregularly shaped shards of AA bone particles may have been "uncomfortable" surfaces for cells to attach upon. This has similarly been observed in unpublished *in vitro* biocompatibility studies. Furthermore, the more densely packed the particles, the less polymer exists in an implant, tending to accelerate the degradation rate. The incorporation of porous particles such as hydroxyapatite, probably contribute to faster degradation both by virtue of there being less polymer and by the ability to hold absorbed fluid within its own sponge-like channels.

3) A variety of factors affect polymer degradation, including molecular weight of the polymer, molar ratio of the polymer constituents, degree of crystallinity, polymer porosity, structural form of the implant and anatomic location. The effect of functional stress on the implant is of prime interest. Further studies of the interrelationship of these parameters are required in order to both

understand and accurately predict the biological behavior of a given polymer implant .

SUMMARY

Flat disks composed of biodegradable poly (DL, lactide-co-glycolide) were implanted into the pectoralis muscles of rats to study the relationship of implant biodegradation to the attachment of cells and tissue matrix. The results of this *in vitro* SEM study suggest that the incorporation of particulate material such as demineralized bone, or hydroxyapatite may contribute to an accelerated rate of polymer degradation. As degradation of the polymer proceeds, there seems to be less direct cell attachment as the material becomes surrounded by a collagenous matrix.

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LEGEND

1. Autolyzed, antigen-extracted bone paricles from Macaca fascicularis. Particles are 200 to 600µm . Original magnification X50.

2. Porous hydroxyapatite particles (Interpore 200). Particles are approximately 1mm diameter with 0.1mm pores. Original magnification X21.

3. Surface of polymer implant one week post-implantation having focal aggregates of erythrocytes and leukocytes in a fibrin clot. a. Original magnification X500; b. magnification X1500.

4. Platelets adhering directly on areas of polymer implant having a glassy smooth surface. a. Original magnification X 1000; b. magnification 1500.

5. Cross-section of plain copolymer implant prior to implantation showing internal porosity with average pore diameter of 0.15µm. Original magnification X 350.

6. Cross-section of copolymer implant one week after implantation. Internal pore size has increased dramatically due to fluid absorption and internal clefting of polymer is apparent. Original magnification X 400.

7. Early degradation of the one week post-implantation sample causes irregular cratering of the polymer surface. Original magnification X17.

8. Degradation at two weeks causes the polymer to develope a globular, cottage-cheese like appearance. Attachment of cells to such exposed areas is minimal. a.Original maginification X250; b. magnification X700.

9. At three weeks post-implantation, the inner portions of the polymer have become fibrinous in appearance, and friable in texture. Orig. magnification X400.

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10. At three weeks, hydroxyapatite particles are exposed (a), however, there is a lack of significant cell attachment to the HA particle or the adjacent polymer (b). a. Original magnification X20; b. magnification X140.

11. Exposure of AA bone particle at three weeks. The copolymer is becoming fibrinous and there are no cells attached where the bone fragment appears.
Original magnification X175.

12. At three weeks the implant is covered with a thin collagenous capsule making it difficult to differentiate stromal ingrowth from fibrinoid degradation of the polymer. A lack of cellular attachment is apparent. Original magnification X1000.

13. Focal area of three weeks of degradation producing a fibrinous, spongy implant matrix with a notable absence of cell attachment. Original magnification X500.